Short Communication

Solanum commersonii Cytoplasm Does Not Improve Freezing Tolerance in Substitution Backcross Hybrids with Frost-sensitive Potato Species

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ABSTRACT

Solanum commersonii Dun. (cmm) is the most frost hardy wild potato species known, being able to tolerate an acute freezing episode to about -5 C and further acclimate to tolerate -10 C after being exposed to chilling temperatures for several days. Breeding with this species to incorporate its frost-hardiness traits can be accomplished by standard sexual hybridization or protoplast fusion. These methods can result in hybrids that vary in contribution of the cmm plastome. To test the effect of cmm cytoplasm, cytoplasmic substitution backcross hybrids were made with three very frost-sensitive species, *S. brachistotrichum*, *S. cardiophyllum*, and *S. pinnatisectum*, by using *S. commersonii* as the female to make an F_1, then performing repeated backcross (BC) using the sensitive species as males. Relative freezing tolerance (RFT) of all genotypes was assessed by measurement of ion leakage of excised terminal leaflets subjected to a controlled ice nucleation and simulated freeze-thaw stress. Even against the background of these very sensitive species' genomes, the cmm cytoplasm of substitution hybrids promoted insignificant improvement in frost hardiness or ability to acclimate. We conclude that either (1) cmm cytoplasm does not contribute to frost hardiness, or (2) if cmm cytoplasmic frost hardiness genes do exist, they must be epistatic to (depend on the presence of) nuclear hardiness genes for expression.

INTRODUCTION

Solanum commersonii (cmm) has been the subject of a large number of research publications. A search in AGRICOLA on "*Solanum commersonii*" returned 68 publications related to frost tolerance, which could be classified as breeding with sexual hybrids (8), breeding with protoplast fusion hybrids (11), crossing methods (13), organelle DNA genetics (2), and physiology (34).

The particular interest in cmm has been due mostly to its outstanding frost-hardiness characteristics. Side-by-side field comparisons of 2,635 populations including most of the species in the US Potato Genebank rated cmm and *S. acaule*, (acl) as the most hardy species (Vega and Bamberg 1995). Controlled tests in the lab (Palta et al. 1981) show that cmm has similar RFT to acl at about 4 to 5 C below freezing, but when these species are frost acclimated (exposed to 12 days of temperatures slightly above freezing), cmm gains about another 5 C of freezing tolerance, while acl gains only about 2 C. When

fully acclimated, cmm can have an RFT of -10 C, which is 3 to 4 C colder than other hardy potato species. Furthermore, unlike other hardy species, cold acclimation in cmm occurs rapidly to a high level and continues throughout the induction period (Vega et al. 2000). Hijmans et al. (2003) used field data on 87 potato species to assign a percentile value for frost hardiness. In that analysis, cmm received a score of 99%.

Several diploid species from Mexico are both exceptionally sensitive to frost and, unlike most South American diploid species, cross readily with cmm to produce diploid progeny with a reasonable level of fertility (Stone et al. 1993). Hijmans (2003) assigned the Mexican species used as frost-sensitive standards in our experiment the following scores: *S. brachistotrichum* (bst) = 23%, *S. cardiophyllum* (cph) = 22%, and *S. pinnatisectum* (pnt) = 20%. These Mexican species were even more sensitive than the 37 species assessed in Series Tuberosa (the taxon of wild species most closely related to commercial cultivars), which had an average score of 32%.

The cytoplasm of plant cells changes due to low temperature stress, both in ultrastructure and composition (Li and Palta 1978; Palta et al. 1993; Chen et al. 1999). If such changes can influence tolerance of frost stress and are regulated by variable cytoplasmic genetics, it would be important to consider the cytoplasmic contribution of parents when breeding for frost hardiness. In potato, *S. commersonii* chloroplast DNA has been shown to be distinct from that of Mexican diploid species (Spooner and Castillo 1997). Some evidence suggests that such differences might be linked with frost hardiness. For example, the large subunit of Rubisco (a key metabolic enzyme encoded by chloroplast genes) from the frost hardy species *S. commersonii* has been shown to be more stable to freeze-thaw cycles than that from *S. tuberosum* (Huner et al. 1981).

Is there empirical evidence that cytoplasm impacts frost hardiness? In cereals, where the most work has been done, evidence has been largely negative (e.g., Limin and Fowler 1984). We are aware of no previous experiments on frost hardiness in potato using substitution backcross hybrids. When Chen et al. (1999) tested six fusion hybrid genotypes, two identified as having only cmm chloroplast DNA and four with only *tuberosum* chloroplast DNA, no differences in frost hardiness or acclimation were evident. That experiment, however, involved a small sample and examined materials that also had a significant cmm component in the genome.

If cytoplasm of cmm contributes to frost hardiness, it would be important to use cmm as the maternal parent in

sexual crossing schemes, especially since it does not have an obvious adverse effect on agronomic tuber traits (Scotti et al. 2003). Or if protoplast fusion hybrids are used, one would want to select regenerants with the cmm plastome. Therefore, we sought to examine the effect of cmm cytoplasm on substitution hybrids with predominantly the frost-sensitive genome of three Mexican diploid species.

MATERIALS AND METHODS

All parental germplasm was obtained from the US Potato Genebank (lead author's address). The F_1 hybrids were made by crossing six individuals of cmm PI 243503 with bulk pollen of six individuals each of bst PI 283095, cph PI 184762, and pnt 186554. Intermediate appearance clearly confirmed progeny as true hybrids. Recurrent backcrossing was conducted by using at least 12 hybrids as females, crossed in the greenhouse or screenhouse with a bulk of pollen from one plant each of the pure sensitive recurrent parent species' populations. Table 1 lists the populations contributing to recurrent parent bulk pollen. Different sensitive populations were used for sensitive BC parents than the contributor to the original F_1 to avoid inbreeding depression. Detailed background information on all PI populations is available online through the NRSP-6, US Potato Genebank web site (http://www.ars-grin.gov/nr6). Since flowers of the hybrids were never observed to produce berries unless hand pollinated, emasculation was not done. Seeds were bulked from all females to represent the next hybrid generation. Each hybrid is not represented by the same number of backcrosses since crossing sometimes completely failed. In such cases, the hybrid generation was preserved clonally as tubers or a new sample of seeds from the most advanced generation was replanted.

The insights potentially obtained from reciprocal BC families had not escaped us—i.e., it would have been interesting to also assess the effect of sensitive species' cytoplasm in a genome of nearly pure frost-hardy cmm genetics. However, this could not be pursued because, in the authors' experience, it is very difficult to produce F_1 hybrids using cmm as the pollen parent.

RFT Evaluation

Each of the three frost-sensitive pure species populations and frost-hardy cmm PI 243503 were grown as controls (one genotype of each of the six bst, cph, and pnt PIs, and six geno-

types of cmm PI 243503). Twice as many (12) BC hybrid seedlings were grown for comparison in case significant segregation and variation was still occurring in these interspecific hybrids, despite being advanced BC generations. Seedlings of all treatments were grown under identical conditions in the greenhouse at the US Potato Genebank. Four nodal cuttings were established for each seedling and transported to the University of Wisconsin Biotron facility at Madison for growth under precisely controlled environmental conditions. Two of each clonal replicate were treated under non-acclimating conditions, and two under acclimating conditions. Non-acclimating treatment consisted of growth in 14-h photoperiod at 20 C day/18 C night with 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR and RH at 70%, and watering with modified half strength Hoagland's solution (Vega et al. 2000). Frost acclimation was induced under conditions previously determined to be optimal: 12 days of 14-h photoperiod at 4 C day/2 C night with 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. RFT was determined by the method described in Vega et al. (2000).

Terminal leaflets were exposed to incrementally freezing temperatures and ion leakage was measured. Percentage mean ion leakage of averaged triplicates was plotted as a function of freezing temperature. RFT value was the degree C below 0 at which the midpoint between maximum and minimum ion leakage occurred.

The various genotypes within a family served as statistical replicates of the treatments and were analyzed with single factor ANOVA. The LSD was calculated (Steele and Torrie 1980:191) and used for the *a priori* comparisons of the three recurrent parent vs BC pairs, as well as cmm to all other means.

RESULTS AND DISCUSSION

Backcross generations were obtained: (cmm x bst) BC$_7$, (cmm x cph) BC$_3$, (cmm x pnt) BC$_4$, which with full recombination and no selection theoretically resulted in nuclear genomes of only 1/256, 1/16, and 1/32 cmm, respectively. For each cross, the general appearance of the BC plants was indistinguishable from the pure recurrent species parent. Table 1 provides results of the freezing tolerance tests for plants both in the non-acclimated and acclimated states.

This experiment verifies cmm's non-acclimated and acclimated RFT of -5 and -10 C, respectively, reported by previous studies (Palta et al. 1981; Chen et al. 1999). The (cmm x cph) hybrid was the only BC family significantly more hardy than its pure recurrent species parent. This may be explained by the fact that some cph are extremely sensitive, reducing that mean, not because the BC hybrid was remarkably hardy (note that the [cmm x cph] BC is actually less hardy than pure bst and pnt, and is much less hardy than pure cmm). The relative hardiness of the (cmm

TABLE 1—*Summary of freezing tolerance (RFT[1]) of sensitive recurrent species, S. commersonii, and BC hybrids.*

Treatments[2]	Number of genotypes	—Non Acclimated RFT—			——Acclimated RFT——		
		Max	Min	Average	Max	Min	Average
bst	5	2.2	1.8	1.97	3.3	2.4	2.74
(cmm x bst) BC$_7$	12	2.3	1.7	2.00	3.0	2.3	2.70
	diff			0.03 ns			0.04 ns
cph	6	2.1	0.8	1.50	3.0	2.2	2.71
(cmm x cph) BC$_3$	12	2.7	1.2	1.93	3.4	2.3	3.10
	diff			0.43 *			0.40 *
pnt	6	2.4	1.8	2.18	3.4	2.4	3.18
(cmm x pnt) BC$_4$	11	2.5	2.1	2.08	3.5	2.4	3.03
	diff			0.10 ns			0.16 ns
cmm	5	6.0	5.1	5.46	10.5	9.7	10.24
	diff[3]			3.28-3.96 **			7.06-7.54 **
LSD$_{0.05}$				0.33			
LSD$_{0.01}$				0.44			

[1]Relative freezing tolerance: The degree C below 0 at which the midpoint between maximum and minimum ion leakage occurs.
    Acclimation = 12 days of exposure to cold but non-freezing temperatures (14-h photoperiod at 4 C day/2 C night).
[2]Representing cmm = plants from PI 243503
    bst = one plant each from PI 255527, 255528, 320265, 498216, 498217
    cph = one plant each from PI 184767, 184771, 275213, 275214, 275215, 279303
    pnt = one plant each from PI 184774, 186553, 230489, 253214, 275230, 275231
[3]ns = not significant (p of diff by chance >5%), * = significant (p of diff by chance: 1% < p < 5%), ** = highly significant (p of diff by chance < 1%). For cmm, range corresponds to comparisons to all treatments above.

x cph) BC may also be due in part to the fact that this hybrid family was derived from relatively few recurrent backcrosses (i.e., BC_3).

All other differences between pure sensitive species and their cmm BC families were small and insignificant, whether with respect to non-acclimated or acclimated RFT, and all families were much (highly significantly) less frost hardy than pure cmm (Table 1).

Thus even against the background of these very sensitive species' genomes, the cmm cytoplasm of substitution hybrids promoted no practical improvement in frost hardiness or ability to frost acclimate. We conclude that either (1) cmm cytoplasm does not contribute to frost hardiness, or (2) if cmm cytoplasmic frost hardiness genes do exist, they must be epistatic to (depend on the presence of) nuclear hardiness genes for expression.

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